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THE EFFECT OF ROUNDUP ON THE BIVALVE *UNIO TUMIDUS* MOLLUSK UTILIZING *EX VIVO* APPROACH

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Glyphosate is the worldwide used herbicide of the first priority. However, its biochemical effects in the aquatic animals are studied scantly. The *ex vivo* approach has been recently proposed to provide the express evaluation of the adverse impact without the treating of the organisms. The aim of this study was to verify this approach for the assessment of the toxicity of glyphosate to the bivalve mollusk. The samples of the gills and digestive gland tissues of freshwater bivalve *Unio tumidus* mollusk were exposed to a range of the concentrations of glyphosate (commercial formulation Roundup MAX) at the concentrations 13.3, 26.7, 66.8 and 133.6 $\mu g \cdot L^{-1}$ during 2 h at 20 °C followed by 15 h at ~ 2–4° C. The markers of oxidative injury (total antioxidant activity, end-products of lipid peroxidation (TBARS) and protein carbonyls (PC)), cellular low weight thiols GSH/GSSG and metallothionein (MT), and cholinesterase activity as the index of neurotoxicity were analyzed. We also assayed the index of cell vitality as the lysosomal membrane stability from the Neutral Red Retention (NRR) test. The results have shown that the lowest concentrations of glyphosate caused the most prominent changes of the indices: the decrease of MT concentration (by ~ two times) and cholinesterase activity. The total antioxidant activity was decreased substantially in all exposures correspon-

dingly to a decrease of the MT and/or GSH concentrations. However, the levels of TBARS and PC were not changed comparing to control detecting the early stage of the injury. Surprisingly, NRR increased in the exposures to higher concentrations of glyphosate, probably due to strong chelating ability of glyphosate or other compounds of formulation. This study allows us to detect the earlier biological effects of glyphosate in the low environmentally realistic concentrations. Further validation of this approach needs the comparison of the results in the *ex vivo* and *in vivo* experiments.

Keywords: Roundup; bivalve mollusk; cytotoxicity; *ex vivo*

INTRODUCTION

Glyphosate (Gl), the modified aminoacid and the organophosphonate compound, N-(phosphonomethyl)glycine, is the most sprayed and most used herbicide in the world [4; 19]. Whereas it acts as the specific inhibitor of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase intrinsic for the plant and bacteria, blocking the synthesis of aromatic compounds, it was originally marketed as absolutely non-toxic for the animals [26]. However, its by-side effect on the non-targeted organisms were reported later: the loss of the immune defense of the animals and human due to the impact on the symbiotic microorganisms [25]. Besides, the strong chelating properties were found for Gl, that cause the immobilization of mineral nutrients by the organisms. Furthermore, in the manufacturing preparations, for example, the Roundup, the active substance was found to inhibit the cytochrome P450 enzymes. Plural effects of Gl and its commercial formulations on the reproductive system, developmental abnormalities and carcinogenic effects in the non-targeted organisms were reported [26]. The biochemical responses that serve for the early warning of ecotoxicity, are less known [18].

The *in vitro* approaches, such as the use of cell lines, primary cultures and reporter gene assays, have been successfully applied to test the toxicity of environmental chemicals [12]. The developed *ex vivo* approach seems to be more biologically relevant comparing to isolated cells due to the maintained natural inter-cellular interactions [14]. The experiments with the tissues of mollusks had started only recently [28; 17], despite these organisms are among the most frequently used in aquatic toxicology to detect adverse impacts in the environment.

The objective of this study was to verify express-method for the assessment of the early signs of the toxicity of Gl to the bivalve mollusk. The biomarkers of oxidative stress responses and the cytotoxicity were evaluated. The concentrations of Gl were corresponding to the environmentally realistic.

MATERIALS AND METHODS

Adult *Unio tumidus* Philipson, 1788 (Unionidae) mollusks (~ 6 years old, 8±1 cm length, and 42±5 g weight) were collected at the confirmed as relatively undisturbed in our previous studies site [15]. The bivalves were collected in autumn season. After period of acclimation in aerated dechlorinated softened tap water (14 days), mollusks were dissected. The samples of digestive gland and gills were maintained at temperature -40 °C till exposure (no more than a week). The modified *ex vivo* approach of El Haj et al. [17] was used. Selection of Gl concentration in the present study was based on

environmentally relevant concentrations of this herbicide. For example, according to environmental quality standards of Brazilia, the maximum value allowed for Class I waters for glyphosate is 65 μg/L [17]. Environmentally realistic concentrations of Gl in Europe corresponding to dozens and hundreds of $\mu g \cdot L^{-1}$ [7]. We treated isolated tissues of *U. tumidus* mussels with organophosphonate pesticide glyphosate (Gl, formulation Roundup MAX, Monsanto, USA) at concentrations 34, 68, 169 and 338 $\mu g \cdot L^{-1}$ (corresponding to 13.3, 26.7, 66.8 and 133.6 $\mu g \cdot L^{-1}$ of Gl) during 2 h at 20 °C followed by 15 h at around 2 °C. Basing on pilot exposures and skepticism concerning quality of samples after long time in warm conditions, we reduced the time of exposure at 20 °C compare to conditions proposed by El Haj et al [17] (15 h). For each exposure, pairs of samples were taken from six mollusks.

Samples were placed into the experimental solution of Gl in Ringer's solution for mollusks (in $g \cdot L^{-1}$, 8 NaCl, 0.2 KCl, 1.4 Na_2HPO_4 and 0.272 KH_2PO_4 (pH 7.4)). Gill tissue was utilized for glutathione (GSH/GSSG) and lysosomal stability (NRR) assays due to its direct contact with water. Gills of mollusks are highly sensitive target for neurotoxic effects, therefore we utilized them for determining of cholinesterase (ChE) activity [5]. The digestive gland was analyzed for oxidative injury as the main metabolic active tissue.

Methodology used for detection of end-products of lipid peroxidation (thiobarbituric acid-reactive substances, TBARS) and protein carbonyls (PC), metallothioneins (MTs), GSH and GSSG concentrations, ChE activity was described in our previous works [15; 16]. Lipid peroxidation (LPO) was determined by TBARS production [21] and referred to fresh weight of tissue (FW). A molar extinction coefficient of $1.56 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$ was used. Protein carbonyl (PC) content, as an index of protein oxidation, was measured by reaction with 2,4-dinitrophenylhydazine (DNPH) [24]. The absorbance was determined by spectrophotometry at 375 nm, and amount of PC was calculated by using a molar extinction coefficient of $2.2 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$. The results were expressed as nmol PC·per g of FW. Total glutathione (GSH) and oxidized glutathione (GSSG) concentrations were quantified by glutathione reductase recycling assay [3]. To estimate GSSG level, protein free sample was treated with 2-vinylpyridine prior to assay. The rate of 5-thionitrobenzoic acid formation from 5,5-dithiobis-2-nitrobenzoate (DTNB) was detected spectrophotometrically at 412 nm. Cholinesterase (ChE, EC 3.1.1.7) activity was determined according to the colorimetric method of Ellman et al [9] with utilizing acetylcholine iodide as substrate. The rate of thionitrobenzoate production evaluated at 412 nm was used to estimate hydrolysis. Enzyme activity was calculated using a molar extinction coefficient of $13.6 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$ and referred to FW.

Total antioxidant/ABTS radical scavenging activity of tissues was determined according to Re et al. [23]. $ABTS^{\bullet+}$ radicals were pre-generated by potassium persulfate. The ascorbic acid was used as the reference compound. The reduction in absorbance was recorded at 734 nm. Obtained result was compared with control (ABTS solution).

Index of cell vitality as lysosomal membrane stability was detected from the Neutral Red Retention (NRR) test, as it was described by El Haj et al [17]. Briefly, tissues were incubated with neutral red for 2 h to allow for uptake of supravital dye into the lysosomes of viable cells. Then samples were washed with saline and fixed in formaldehyde (0.5% in 1% $CaCl_2$) for 1 h, then freezed during 30 h. After that, the dye was extracted in acid alcohol (1% acetic acid in 50% ethyl alcohol) and measured at 550 nm.

All measurements were carried out in 12 samples from 6 specimens in each group. The results were expressed as means ± standard deviation (SD). The reliability of deviation of two series was calculated using the Student's *t*-test. Difference between groups was considered reliable at $P<0.05$. All statistical calculations were performed with Statistica v 12.0 and Excel for Windows-2000.

RESULTS AND DISCUSSION

High sensitivity of responses in the isolated tissues to effect of Roundup was revealed. Total antioxidant activity in the digestive gland was most affected (Fig. 1***A***). It was reduced by about 30 % comparing to control at all exposures. However, in the studied time limits, that was not realized in the enhanced level of the end-products of the oxidative injury (TBARS and PC) detecting early stage of injury (Fig. 1***B***, ***C***). Only exposure to 66.8 μg·L^{-1} of Gl increased significantly ($P<0.04$), but not prominently level of PC in digestive gland.

The concentration of GSH in the gills was decreased by the highest concentrations of Gl by 1.6 times. It was accompanied by the increased oxidation of this thiol (by two times) with the reducing of the Redox Index of GSH (GSH/GSSG) from 3.5 to 1.7 at the highest concentration of Gl (Fig. 2***A***, ***B***).

The metallothioneins of the digestive gland were most targeted at low concentrations of Gl with the decreasing by 1.8 times (Fig. 2***C***). Similarly, the cholinesterase activity in the gills was also changed at low concentration of Gl with the decrease by 21% (Fig. 3***A***). The determining of lysosomal membrane stability in the gills gives the unusual results: it was increased four times at the exposure to the highest concentration (Fig. 3***B***).

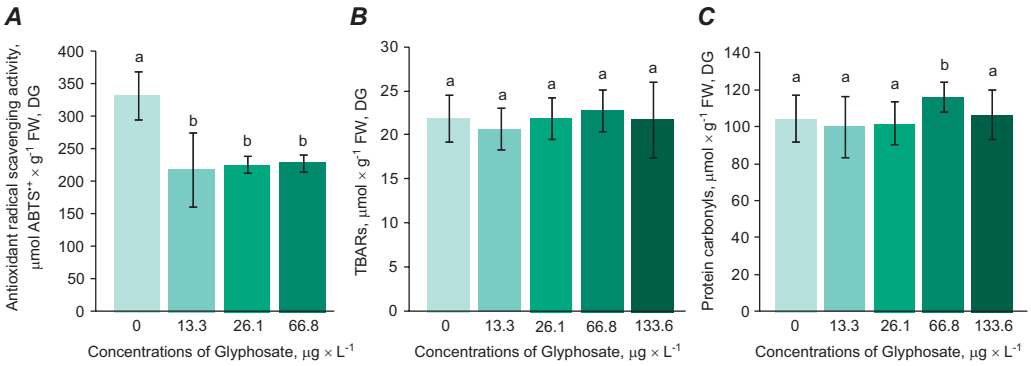

Fig. 1. The oxidative stress indices in the digestive gland of the bivalve mollusk under the *ex vivo* exposures. Data for ***A*** – total antioxidant activity; ***B*** – TBARS; ***C*** – protein carbonyls (PC), presented as mean ± SD (N = 6). Here and in Fig. 2,3 if the letters above the bars are the same, this indicates that the values do not differ significantly ($P > 0.05$)

Рис. 1. Показники окисного стресу у травній залозі двостулкових молюсків під впливом гліфосату *ex vivo*: ***A*** – загальна антиоксидантна активність; ***B*** – ТБК-активні продукти; ***C*** – окисні модифікації протеїнів, представлені як середнє значення ± SD (N = 6). Тут і на рис. 2, 3, якщо букви над стовпцями однакові, це означає, що значення несуттєво відрізняються ($P> 0,05$)

These results demonstrate high sensitivity of this approach. In the study of El Haj et al. [17], 24, 240, 2400 and 24,000 μg·L^{-1} of Gl (in the composition of Roundup

Transorb® formulated product) revealing the *ex vivo* cytotoxicity at the exposures to 24 and 2,400 μg·L^{-1} of GI in the tissues of the bivalves.

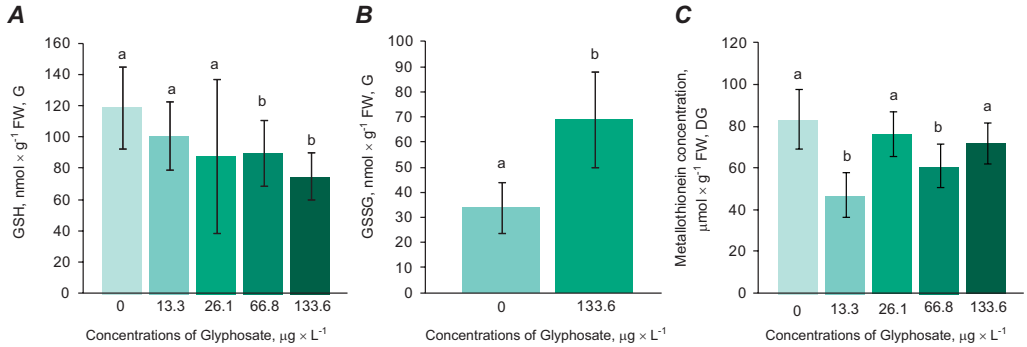

Fig. 2. Cellular low weight thiols in the digestive gland (DG) or gills (G) of the bivalve mollusk under the *ex vivo* exposures to glyphosate. Data for ***A*** – GSH (G); ***B*** – GSSG (G); ***C***: – metallothionein (DG), presented as mean ± SD (N=6)

Рис. 2. Клітинні низькомолекулярні тіоли у травній залозі (Т3) або зябрах (З) двостулкових молюсків під впливом гліфосату *ex vivo*. ***A*** – GSH (З); ***B*** – GSSG (З); ***C*** – металотіонеїни (Т3), представлені як середнє значення ± SD (N = 6)

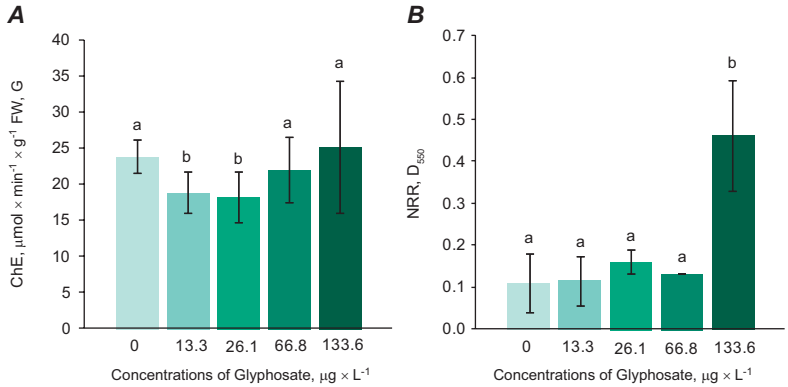

Fig. 3. The indices of toxicity in the gills of the bivalve mollusk under the *ex vivo* exposures to glyphosate. Data for ***A*** – cholinesterase (ChE) activity; ***B*** – the lysosomal membrane stability (NRR), presented as mean ± SD (N = 6)

Рис. 3. Показники токсичності у зябрах двостулкових молюсків під впливом гліфосату *ex vivo*: ***A*** – активність холінестерази (ChE); ***B*** – стабільність лізосомальних мембран (NRR), представлені як середнє значення ± SD (N = 6)

Despite GI is one of the most used herbicide worldwide, its biological effects are poorly investigated [18]. The methodology applied in our study confirms the suitability of *ex vivo* approach and strengthens it due to application of low environmentally realistic concentrations and widening a set of the biomarkers. The oxidative effect of GI was proved. Similar to utilized concentrations of GI caused the same response *in vivo* in freshwater amphipod *Gammarus pulex* during the acute exposure (decreased level of GSH and increased level of TBARS) [22]. In our study, the metallothioneins appeared to be most vulnerable thiol at low concentration of GI followed by the GSH, that was

depleted at higher concentrations of GI. Probably, a depletion of the metallothionein and GSH can explain a decrease in total antioxidant scavenging activity, whereas GSH and metallothioneins enable to neutralize free radicals, especially the reactive oxygen species (ROS) such as the superoxide, hydroperoxyl, hydroxyl ($OH^{\bullet}$) radicals, having electron acceptor ability [12]. An increased production of the GI-mediated oxyradicals was suspected in exposures [2].

In present study, an impact on the cholinergic system was shown with a decrease of cholinesterase activity. Despite GI is a organophosphorus compound that do-not belong to the organophosphates, typical inhibitors of cholinesterase, like chlorpyrifos [5; 6], its neurotoxic effect was evident. It was also indicated in the exposures of *G. pulex* [22] and *Mytilus galloprovincialis* [18].

We detected increased retention of the dye in the lysosomes under the exposures to higher GI concentrations. This response is opposite to typical permeabilization of lysosomal membranes under the toxic exposures [17]. We explain this manifestation by strong chelating ability of GI or other compounds of the formulation [20]. Presence of surfactants in commercial formulations modulated effect of GI for juvenile oysters (*Crassostrea gigas*) [25]. Roundup MAX formulation contains surfactants POEA (polyethoxylated tallow amines). As a wetting agents, they can cause membrane permeability [8]. However, some surfactants, like poloxamer 188, cause an increase in membrane stability, opposite to sodium dodecyl sulphate lowering the interactions between phospholipid molecules due to its incorporation in cellular membranes [27]. The strengthening of the lysosomal membranes was also shown for human fibroblasts with cholesterol-loaded lysosomes [1]. In our study, cholesterol content was able to change the membrane stability more than three times. In *ex vivo* study of El Haj et al. [17], NRR test had shown a decrease of retention under 50 and 5,000 $\mu g \cdot L^{-1}$ concentrations of the Roundup Transorb. Regarding an increased release of the dye from lysosomes at lower concentration of GI, it was also observed by El Haj et al. [17]. That might be explained by extremely long time of exposition of the dissected tissue under 15 h treatment at 20 °C. However, the absence of the effect at 500 $\mu g \cdot L^{-1}$ of substance raises skepticism concerning reasons for this loss of vitality in the study [17].

CONCLUSION

In this study, *ex vivo* approach was used to detect early biological effects of GI in low environmentally realistic concentrations. However, long-term exposure of the organism to environmentally realistic concentrations of GI can cause particular responses, including the adaptation to this xenobiotic. Further validation is needed to compare of the results of *ex vivo* and *in vivo* experiments.

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1. *Appelqvist H., Sandin L., Björnström K., Saftig P., Garner B., Öllinger K., Kagedal K.* Sensitivity to lysosome-dependent cell death is directly regulated by lysosomal cholesterol content. **PloS One**, 2012; 7(11).
[DOI: http://dx.doi.org/10.1371/journal.pone.0050262; PMid:23166840; Google Scholar]
2. *Akcha F., Spagnol C., Rouxel J.* Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos. **Aquat. Toxicol.,** 2012; 106: 104–113.
[DOI: https://doi.org/10.1016/j.aquatox.2011.10.018; PMid:22115909; Google Scholar]
3. *Anderson M.E.* Determination of glutathione and glutathione disulfide in biological samples. **Meth. Enzymol,** 1985; 113: 548–555.
[DOI: https://doi.org/10.1016/S0076-6879(85)13073-9; Google Scholar]
4. *Annett R., Habibi H.R., Hontela A.* Impact of glyphosate and glyphosate-based herbicides on the freshwater environment. **J. Appl. Toxicol.,** 2014; 34(5): 458–479.
[DOI: https://doi.org/10.1002/jat.2997; PMid:24615870; Google Scholar]
5. *Beltran K.S., Pocsidio G.N.* Acetylcholinesterase activity in *Corbicula fluminea Mull*., as a biomarker of organophosphate pesticide pollution in Pinacanauan River, Philippines. **Environ. Monit. Assess.,** 2010; 165: 331–340.
[DOI: https://doi.org/10.1007/s10661-009-0949-y; PMid:19444631; Google Scholar]
6. *Bianco K., Yusseppone M.S., Otero S., Luquet C., de Molina M.D.C.R., Kristoff G.* Cholinesterases and neurotoxicity as highly sensitive biomarkers for an organophosphate insecticide in a freshwater gastropod (*Chilina gibbosa*) with low sensitivity carboxylesterases. **Aquat. Toxicol.,** 2013; 144: 26–35.
[DOI: https://doi.org/10.1016/j.aquatox.2013.09.025; PMid:24140633; Google Scholar]
7. *Carles L., Gardon H., Joseph L., Sanchís J., Farre, M., Artigas J.* Meta-analysis of glyphosate contamination in surface waters and dissipation by biofilms. **Environ. Int.,** 2019; 124: 284–293.
[DOI: https://doi.org/10.1016/j.envint.2018.12.064; PMid:30660841; Google Scholar]
8. *Czarnota M., & Thomas P.A.* Using surfactants, wetting agents, and adjuvants in the greenhouse. **University of Georgia J.,** 2010.
[URL: http://hdl.handle.net/10724/12373]
9. *Ellman G.L., Courtney K.D., Andres V.J., & Featherstone R.M.* A new and rapid colorimetric determination of acetylcholinesterase activity. **Biochem. Pharmacol.,** 1961; 7(2): 88–95.
[DOI: https://doi.org/10.1016/0006-2952(61)90145-9; Google Scholar]
10. *Falfushynska H., Gnatyshyna L., Stoliar O.* In situ exposure history modulates the molecular responses to carbamate fungicide Tattoo in bivalve mollusk. **Ecotoxicology,** 2013; 22 (3): 433–445.
[DOI:https://doi.org/10.1007/s10646-012-1037-6; PMID: 23306937; Google Scholar]
11. *Fent K.* Fish cell lines as versatile tools in ecotoxicology: assessment of cytotoxicity, cytochrome P4501A induction potential and estrogenic activity of chemicals and environmental samples. **Toxicol. In Vitro,** 2001; 15(4-5): 477–488.
[DOI: https://doi.org/10.1016/S0887-2333(01)00053-4; Google Scholar]
12. *Fiser B., Jójárt B., Csizmadia I.G., Viskolcz B.* Glutathione – hydroxyl radical interaction: a theoretical study on radical recognition process. **PLOS ONE**, 2013; 8(9).
[DOI: https://doi.org/10.1371/journal.pone.0073652; PMid:24040010; Google Scholar]
13. *Giuliani M.E., Sparaventi E., Lanzoni I., Pittura L., Regoli F., Gorbi S.* Precision-Cut Tissue Slices (PCTS) from the digestive gland of the Mediterranean mussel *Mytilus galloprovincialis*: An *ex vivo* approach for molecular and cellular responses in marine invertebrates. **Toxicol. In Vitro,** 2019; 61.
[DOI: https://doi.org/10.1016/j.tiv.2019.104603; PMid:31330176; Google Scholar]

14. *Gnatyshyna L., Falfushynska H., Mykhalska V., Mischuk N., Stoliar O.* Multi-marker study of the response of bivalve mollusk *Unio tumidus* induced by the compounds of typical municipal effluents. **Studia Biologica,** 2017; 11(2), 37–44.
[DOI: https://doi.org/10.30970/sbi.1102.540; Google Scholar]
15. *Gnatyshyna L., Khoma V. , Horyn O., Ozoliņš D., Skuja A., Kokorite I., Rodinov V., Martyniuk V., Spriņģe G., Stoliar O.* Multi-marker study of *Dreissena polymorpha* populations from hydropower plant reservoir and natural lake in Latvia. **Turk. J. Fish.& Aquat. Sci.**, 2020; 20(6): 409–420.
[DOI: http://doi.org/10.4194/1303-2712-v20_6_01; Google Scholar]
16. *Gnatyshyna L., Khoma V., Mishchuk O., Martinyuk V., Spriņģe G., & Stoliar O*. Multi-marker study of the responses of the *Unio tumidus* from the areas of small and micro hydropower plants at the Dniester River Basin, Ukraine. **Environ. Sci. Pollut. Res.,** 2020; 1–12.
[DOI: https://doi.org/10.1007/s11356-020-07698-4; PMid:31955329; Google Scholar]
17. *Haj E.I.Y., Bohn S., Souza M.M.* Tolerance of native and invasive bivalves under herbicide and metal contamination: an *ex vivo* approach. **Environ. Sci. Pollut. Res.,** 2019; 30: 31198–31206.
[DOI: https://doi.org/10.1007/s11356-019-06256-x; PMid:31463750; Google Scholar]
18. *Matozzo V., Fabrello J., Masiero L., Ferraccioli F., Finos L., Pastore P., Di Gangi I.M., Bogialli S.* Ecotoxicological risk assessment for the herbicide glyphosate to non-target aquatic species: a case study with the mussel *Mytilus galloprovincialis*. **Environ. Pollut.,** 2018; 233: 623–632.
[DOI: https://doi.org/10.1016/j.envpol.2017.10.100; PMid:29107902; Google Scholar]
19. *Mensah P.K., Palmer C.G., Odume O.N*. Ecotoxicology of glyphosate and glyphosate-based herbicides-toxicity to wildlife and humans. In: Larramendy M.L., Soloneski S. (Ed.) **Toxicity and Hazard of Agrochemicals**. InteachOpen, 2015: 93–111.
[DOI: https://doi.org/10.5772/60767; Google Scholar]
20. *Mertens M., Höss S., Neumann G., Afzal J., Reichenbecher W*. Glyphosate, a chelating agent-relevant for ecological risk assessment? **Environ. Sci. Pollut. Res.,** 2018; 25(6): 5298–5317.
[DOI: https://doi.org/10.1007/s11356-017-1080-1; PMid:29294235; Google Scholar]
21. *Ohkawa H., Ohishi N., Tagi K.* Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. **Anal. Biochem.,** 1979; 95: 351–358.
[DOI: https://doi.org/10.1016/0003-2697(79)90738-3; Google Scholar]
22. *Pala A.* The effect of a glyphosate-based herbicide on acetylcholinesterase (AChE) activity, oxidative stress, and antioxidant status in freshwater amphipod: *Gammarus pulex* (Crustacean). **Environ. Sci. Pollut. Res.,** 2019; 26: 36869–36877.
[DOI: https://doi.org/10.1007/s11356-019-06804-5; PMid:31745777; Google Scholar]
23. *Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C.* Antioxidant activity applying an improved ABTS radical cation decolorization assay. **Free Radic. Biol. Med.,** 1999; 26(9–10): 1231–1237.
[DOI: https://doi.org/10.1016/S0891-5849(98)00315-3; Google Scholar]
24. *Reznick A. Z., Packer L.* Oxidative damage to proteins: spectrophotometric method for carbonyl assay. **Meth. Enzymol.,** 1994; 233: 357–363.
[DOI: https://doi.org/10.1016/S0076-6879(94)33041-7; Google Scholar]
25. *Séguin A., Mottier A., Perron C., Lebel J.M., Serpentini A., Costil K.* Sub-lethal effects of a glyphosate-based commercial formulation and adjuvants on juvenile oysters (*Crassostrea gigas*) exposed for 35 days. **Mar. Pollut. Bull.,** 2017; 117(1–2): 348–358.
[DOI: http://dx.doi.org/10.1016/j.marpolbul.2017.02.028; PMid:28202277; Google Scholar]

26. *Torretta V., Katsoyiannis I.A., Viotti P., Rada E.C*. Critical review of the effects of glyphosate exposure to the environment and humans through the food supply chain. **Sustainability,** 2018; 10(4): 950–970.
[DOI: https://doi.org/10.3390/su10040950; Google Scholar]
27. *Tung L., Troiano G.C., Sharma V., Raphael R.M., Stebe K.J*. Changes in electroporation thresholds of lipid membranes by surfactants and peptides. **Ann. N. Y. Acad. Sci.,** 1999; 888(1): 249–265.
[DOI: https://doi.org/10.1111/j.1749-6632.1999.tb07960.x; PMid:10842637; Google Scholar]
28. *Vehovszky Á., Farkas A., Csikós V., Székács A., Mörtl M., Győri J.* Neonicotinoid insecticides are potential substrates of the multixenobiotic resistance (MXR) mechanism in the non-target invertebrate, *Dreissena* sp. **Aquat. Toxicol.,** 2018; 205: 148–155.
[DOI: https://doi.org/10.1016/j.aquatox.2018.10.013; PMid:30384196; Google Scholar]
29. *Viarengo A., Burlando B., Dondero F.* Metallothionein as a tool in biomonitoring programmes. **Biomarkers**, 1999; 4: 455–466.
[DOI: https://doi.org/10.1080/135475099230615; PMid:23902390; Google Scholar]

ВПЛИВ РАУНДАПУ НА ДВОСТУЛКОВОГО МОЛЮСКА *UNIO TUMIDUS* ІЗ ВИКОРИСТАННЯМ ПІДХОДУ *EX VIVO*

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Гліфосат – один із найбільш використовуваних гербіцидів у світі. Він діє як специфічний інгібітор ензиму 5-енолпірувілшикимат-3-фосфат синтази, властивого рослинам і бактеріям, блокуючи синтез ароматичних сполук, проте проявляє і побічні ефекти у тварин, пов'язані з пригніченням імунного захисту й ураженням симбіотичних бактерій. Відомо про його генотоксичність. Біохімічні реакції водяних тварин за його дії досліджені мало. Нещодавно було запропоновано експрес-підхід *ex vivo* до оцінювання несприятливого впливу без ушкодження організмів. Метою нашого дослідження було перевірити цей підхід до оцінювання токсичності гліфосату для двостулкового молюска. Зразки тканини зябер і травної залози прісноводного двостулкового молюса *Unio tumidus* піддавали впливу таких концентрацій гліфосату (препарат Roundup MAX): 13,3; 26,7; 66,8 і 133,6 мкг/л протягом 17 год (2 год за 20 °C та 15 год за ~ 2–4 °C). Нами проаналізовано маркери окисного стресу (загальна антиоксидантна активність, кінцеві продукти перекисного окиснення ліпідів (TBARS) і карбоніли протеїнів (PC)), низькомолекулярні тіоли (GSH/GSSG і металотіонеїн (MT)), а також активність холінестерази як показник нейротоксичності. Ми також визначили індекс життєздатності клітин за стабільністю лізосомальних мембран за допомогою тесту утримання нейтрального червоного (NRR). Результати вказують на те, що найнижчі концентрації глікосату спричиняють найпомітніші зміни показників: зниження концентрації MT (~ удвічі) й активності холінес-

терази. Загальна антиоксидантна активність значно знижувалася за всіх впливів відповідно до зниження концентрації MT та/або GSH. Відтак, за умов *ex vivo* дія препарату гліфосату спричиняє послаблення антиоксидантного потенціалу тканин, пов'язаного з концентрацією клітинних тіолів, і типову для гострotоксичної дії фосфороорганічних сполук нейротоксичність. Однак рівень TBARS і PC не відрізнявся порівняно з контролем, що можна пояснити коротким періодом інкубації тканини. Несподівано показник NRR зростав за дії найвищої використаної концентрації гліфосату, ймовірно, завдяки хелатуючій здатності гліфосату чи інших складових використовуваного препарату. Це дослідження дає змогу виявити ранні біологічні ефекти гліфосату в низьких екологічно реалістичних концентраціях. Подальша перевірка цього підходу потребує порівняння результатів у експериментах *ex vivo* та *in vivo*.

Ключові слова: Раундап; двостулковий молюск; цитотоксичність; *ex vivo*

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